



The coastal zone may be the single most important portion of our planet. The loss of its biodiversity may have repercussions far beyond our worst fears.

G. Carleton Ray, in *Biodiversity* (1988)

MARINE SCIENCE

Diving Deeper into the *Pfiesteria* Mystery

Although the dinoflagellate *Pfiesteria* has not triggered any major U.S. fish kills or human illness since 1998, controversy continues to swirl around the perplexing organism. In the latest challenge to the developing knowledge of the organism, two teams of researchers, with four overlapping members, say that one species of *Pfiesteria* does not kill by emitting a toxin, but by maiming and killing through other methods, possibly in conjunction with other organisms. Their studies were published 5 August 2002 online in *Nature* and in the 20 August 2002 *Proceedings of the National Academy of Sciences* (PNAS). However, an established *Pfiesteria* researcher is highly critical of their methods and conclusions.

The teams investigated the potential toxin-emitting properties of one strain of *P. shumwayae* through several avenues. The PNAS team began by culturing a clonal isolate on both algal prey and tilapia. They then exposed test fish to solutions from the cultures and found that the fish died in about 12–72 hours. They centrifuged those tank waters to separate liquids and other materials—which presumably would have contained any toxin—from the water, and exposed additional test fish to those redissolved substances. None died. They also exposed test fish to solutions that had been freeze-dried, extracted sequentially with dichloromethane and methanol, and redissolved in water, which again would presumably contain any toxin. Again, no fish died.

As additional tests, they used genetic analysis techniques to look for evidence of polyketides,

which are synthesized by a family of enzymes known as PKSs and are the only group of ichthyotoxins known to be produced by dinoflagellates. They found evidence of some PKS-encoding genes, but concluded that these genes are involved in production of polyunsaturated fatty acids, not toxins. Based on these angles of investigation, the team concluded that *P. shumwayae* does not produce a toxin, although principal investigator Robert Gawley, a professor of chemistry at the University of Miami, says other species of *Pfiesteria* could still be producing a toxin.

The *Nature* team used similar methods, as well as other techniques, to expose test fish to waters containing *P. shumwayae*, but did not perform any genetic analysis. They observed relatively low mortality of test fish, up to about 25%, within 24 hours, and 92–100% mortality within

48 hours. Through use of scanning electron microscopy, they concluded that fish died after dinospores fed extensively on their skin. They also concluded that no toxins existed in the test waters, because none passed through membranes that were known to be permeable to several known toxins.

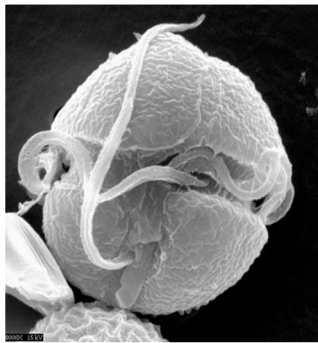
However, JoAnn Burkholder, a North Carolina State University professor of aquatic botany who has studied *Pfiesteria* for more than a decade, isn't surprised the teams found no toxins. "These people have no idea how to culture *Pfiesteria*," she says, "They have never worked with toxic *Pfiesteria*. You can't just throw a fish in a beaker and call it a day."

She points to differences between the peer-reviewed protocols published elsewhere and the methodology used in these studies to culture *Pfiesteria* and to allow expression of toxicity. She says the teams had higher ammonia concentrations and low pH in cultured solutions, and allowed

excessively lengthy time periods for observing mortality. They studied only a single strain of the more than 400 known to exist. She also notes that the toxicity data for *Pfiesteria* have been cross-confirmed by multiple laboratories and published in more than 50 peer-reviewed articles. Several groups have found *Pfiesteria* toxin in pure cultures grown under conditions that allow them to express toxicity, she adds.

When asked if his team's methods were sound, Gawley says, "Yes, in a word." However, he acknowledges that something still unknown is occurring in fish-killing waters. "There's all kinds of stuff in [the water]. It may well be there's a toxin in the cultures the Burkholder group has. My question would be, who made it?"

Many facets of *Pfiesteria*, from its life cycle to its lethality, likely will continue to be controversial until ongoing research at numerous laboratories provides additional information. Tests for the organism continue to turn up positive in waters along the eastern U.S. seaboard and elsewhere in the world. —Bob Weinhold



Change in M.O.? New research suggests that *P. shumwayae* (inset) may kill fish not by releasing a toxin, as previously believed, but by eating their skin.

RECYCLING

Is Sludge Safe?

The U.S. Environmental Protection Agency (EPA) should initiate a hefty research program into the human health risks of using treated sewage sludges ("biosolids") on land, according to a National Research Council committee report released in July. The report, titled *Biosolids Applied to Land: Advancing Standards and Practices*, was requested by the EPA to address public health concerns and to fulfill a Clean Water Act requirement to periodically reassess the science behind the so-called Part 503 rule.

This rule sprang from the Clean Water Act and came into effect in 1993. It established management practices for land application of sewage sludges, concentration limits, loading rates for chemicals, and treatment and use requirements designed to control and reduce pathogens as well as the number of bugs, birds, and rodents that the sludge might attract.

The study identified three major gaps in the scientific basis of the rule: a lack of knowledge about potential human health effects and exposure, a need for an updated risk assessment of chemical contaminants, and a need to assess the risk posed by pathogens in sludge. The committee also found that there is no documented scientific evidence of adverse human health effects from treated sewage sludges applied to land in accordance with the EPA's regulations.

However, according to committee chair Thomas Burke, a public health professor at The Johns Hopkins University in Baltimore, Maryland, this finding was "tempered by the fact that there are few studies available on human exposure to biosolids, and that, even when they are investigated locally, there are no means of tracking health allegations nationally."

Reaction to the report has been favorable from all sides. The EPA and the water and sludge industry welcomed the finding of no proven health problems. Environmental groups and concerned scientists welcomed the research agenda.

The Part 503 rule has been controversial since its inception in 1993. It divides sludges into two classes based on pathogen content. In Class A biosolids, pathogens are below detection levels, whereas Class B biosolids have detectable levels of pathogens. However, the risk from pathogens was never formally assessed for the biosolids

rule, nor has the potential exposure of neighbors to pathogens and contaminants resulting from wind dispersion or runoff been taken into account. Municipalities and counties in some states including California, Virginia, Florida, and New Hampshire have instituted land application bans or restrictions on sludge application.

The increase in land application of biosolids has engendered an increase in health complaints, says Burke. These complaints are anecdotal, but they may be important, he adds.

"I am increasingly convinced that in some places people are getting sick, sometimes very sick, from Class B sludge applications," says Ellen Harrison, director of the Cornell Waste Management Institute in Ithaca, New York. Her organization has tracked down some 40 allegedly sludge-related health incidents affecting over 300 people as of August 2002. Respiratory and gastrointestinal symptoms are most common. Other frequent complaints include nosebleeds, flu-like symptoms, fatigue, and burning eyes, throat, and nose.

To find out whether health effects can be linked to biosolids exposure, the committee recommends that unexpected outbreaks or unusual exposures be studied along with preplanned exposure assessment studies of farmers, sludge workers, and nearby residents. In addition, a few well-designed epidemiological investigations of exposed populations should be conducted to see if there is a causal association between biosolids exposure and adverse health effects.

The committee urges a new survey of contaminants in sludge to include pathogens and organic contaminants of emerging concern such as flame retardants and detergent surfactants. A new risk assessment incorporating probabilistic methods and allowing for regional differences in climate and soils should also be conducted, the committee finds.

The EPA has until April 2003 to craft a response and request public comment, in accordance with a legal agreement from a previous lawsuit related to sludge regulations. Any research program will involve funding across and outside of the agency, says Alan Hais, associate director of the EPA's Health and Ecological Criteria Division, adding that such research will cost millions of dollars. —**Rebecca Renner**

Tightening Trade in Toxics

At the 9th Session of the International Negotiating Committee for the Rotterdam Convention, held in Bonn 29 September–4 October 2002, monocrotophos, an insecticide widely used in developing countries, was added to the list of chemicals whose import can be banned under the convention.

The committee also considered the five forms of asbestos not currently covered by the convention, as well as the



Chrysotile asbestos

pesticides Granox TBC/Spinox T, a fungicide/insecticide mixture used by peanut farmers, and DNOC, used as an insecticide, weedkiller, and fungicide. However, due to questions about how these toxics should be listed, they were not accepted at this time. They are expected to be added at the 10th Session, to be held in November 2003 in Geneva, Switzerland.

Biopirates Beware

On 19 February 2002 12 countries that together contain 70% of the world's plant and animal biodiversity signed an agreement to work toward establishing new trade rules to govern patenting and registering of products based on plant and animal resources. These rules would replace those outlined in the 1992 UN Convention on Biological Diversity. The Group of Allied Mega-Biodiverse Nations—made up of Brazil, China, Colombia, Costa Rica, Ecuador, India, Indonesia, Kenya, Mexico, Peru, South Africa, and Venezuela—believes the convention's wording allows too many loopholes, and that it has not been effectively implemented by the countries that have ratified it. The group also wants to ensure that member countries and the indigenous tribes living within them receive equitable compensation and benefits for resources found on their lands.

Green Grants to Developing Countries

The Global Greengrants Fund (GGF) and Pesticide Action Network (PAN) announced in March 2002 they would award an additional \$120,000 in GGF grants to new or small community-based environmental and social organizations in the most underserved and threatened developing countries. Such groups, which often have little outside financial support, can often achieve a great deal with limited resources. Since 1999, PAN has partnered with GGF to help it award over \$210,000 to 39 groups. Recipients have implemented projects including surveys and workshops on the impacts of persistent organic pollutants, integrated pest management training, and educational materials on pesticides and genetically engineered plants.



BUILT ENVIRONMENT

Rooftop Gardens a Cool Idea

Rooftop gardens are blooming across North America! Rooftop gardens may combat the phenomenon of city air temperatures rising 2–10°F higher than surrounding nonurban areas because of the abundance of dark, heat-absorbing surfaces—such as, well, rooftops. These “urban heat islands” increase the demand for air conditioning, which increases power plant emissions of pollutants that promote global warming. Heat islands also increase ground-level ozone, formed when heat, sunlight, and chemicals in the air mix. Ozone causes coughing and worsens symptoms of asthma, emphysema, and lung cancer.

The idea is simple—roofs are lined with a drainage layer, a waterproof membrane, a growing medium, and, of course, plants. The plants cool the air when water transpires from leaf surfaces and evaporates into the surrounding air. They help shade the soil surface, thus reducing heat buildup in the substrate materials. They also absorb air pollutants such as carbon monoxide, storing them in intracellular spaces, and provide thermal insulation.

Jessica Rio, who is public information officer for the Chicago Department of

Environment, says the air on the rooftop garden atop Chicago's city hall is 15–20°F cooler than the air on the tar roof next door. “This building doesn't have to work as hard to be cool,” Rio says. If reducing a heat island reduces a region's overall temperature, it could also mean less work for air-conditioning units in every building in the area.

Canada's federal National Research Council (NRC) has reached similar conclusions. In research presented at the Green



Temps go down up on the roof. Rooftop gardens offer a natural way to cool buildings in urban settings.

Roof Workshop, held 5 March 2002 in Vancouver, British Columbia, NRC research officer Karen Liu reported that the rooftop garden at the NRC campus in Ottawa, Ontario, reduced the heat entering the building through the roof alone by as much as 85% on summer days.

Whether that decrease translates into significantly better energy efficiency overall depends on the building's construction, in particular the roof-to-wall ratio, says Liu. “In a high-rise commercial building with lots of windows and walls, the energy lost through those openings will play a bigger role in energy efficiency,” she says. “But in a warehouse-type building with a large roof and few windows and walls, the energy saved could be great.”

Green roofs also absorb rainfall. For that reason, rainy Portland, Oregon, provides financial incentives for builders to install rooftop gardens. Tom Liptan, a city environmental specialist, says the city's experimental rooftop garden, installed on an apartment building in 1999, absorbs up to 1 inch of rainfall at a time in its 2- to 6-inch depth of soil. “That's a real nice chunk of water,” he says. The garden roof also reduces runoff by as much as 90% compared with regular roofs. Slowing down drainage of stormwater into combined sewer systems reduces the amount of sewage that overflows into rivers and lakes, he says.

One drawback of rooftop gardens is that they initially cost more than traditional roofs. But the initial cost is offset by the fact that garden roofs last longer, because they are shielded from ultraviolet radiation, and because they don't experience the dramatic temperature fluctuations that can damage traditional roofing material.

—Angela Spivey

GENETICS

Unraveling an Inherited Disorder: AATD

Alpha-1 antitrypsin deficiency (AATD) is an inherited genetic disorder leading in some patients to potentially fatal lung or liver disease. AATD is known to have a particularly strong environmental component; those affected must avoid environmental chemicals, especially cigarette smoke, and all particulates, which accelerate deterioration of lung function. Until recently, AATD was thought to occur mainly in people of northern European descent; it is actually found among many different ethnicities, including African blacks, Arabs, Middle Eastern Jews, Asians, and whites. These and other important facts were presented in a one-day workshop held 19 August 2002, cosponsored by the NIEHS, the NIH Office of Rare Disease Research, and the Alpha-1 Foundation, a patient advocacy group based in Miami, Florida.

The condition has been diagnosed in more than 5,000 people in the United States, but the Alpha-1 Foundation estimates that there are approximately 70,000 U.S. “Alpha-1s.” The only current treatment for AATD is weekly infusion with a human serum-derived substitute AAT protein that is in chronically short supply.

NIEHS genetic epidemiologist Frederick de Serres, himself an Alpha-1, outlined his ongoing research into the worldwide racial and ethnic distribution of the gene alleles associated with AATD. In the 58

countries where genetic epidemiological surveys for AATD have been conducted, there are 117 million carriers, 3.4 million of whom carry the two alleles most associated with risk—a much more widespread distribution than previously believed. In the United States alone there are 24.6 million carriers, 600,000 of whom carry the two alleles most associated with risk.

Robert Sandhaus, clinical director of the Alpha-1 Foundation, said studies of the Alpha-1 community may well identify environmental and genetic risk factors that apply to the general population at risk for chronic lung or liver disease. The foundation keeps a research registry of more than 2,000 patients, a subgroup that is well characterized genetically, willing to participate in studies, and especially sensitive to environmental exposures. But disease registries have inherent biases based on socioeconomic, racial, and health status factors, so researchers need to identify a nonbiased population to more effectively validate AATD studies.

As for other future research directions, Sandhaus said the epidemiology of AATD and its natural history still is in need of a great deal of further research. Priority should also be placed on learning more about the role of environmental exposure sensitivities, identifying early biomarkers of disease and exposure sensitivity, and isolating additional genetic factors, such as modifiers that affect the adverse expression of the most common alleles, both in the same family and among different ethnic subgroups. In addition, says de Serres, researchers must find a cure. —Ernie Hood



Global Biodiversity Information Facility

In 1996, the Megascience Forum Working Group on Biological Informatics of the Organisation for Economic Co-operation and Development (OECD) determined that, although research has gathered a wealth of biodiversity and ecosystem information, the information is so scattered and inaccessible that its utility is wasted. So in 2001, the Global Biodiversity Information Facility (GBIF) was established at the University of Copenhagen to serve as an international mechanism to make this information widely and freely available to anyone with Internet access. One tool it uses to do this is its website, located at <http://www.gbif.org/>.

The primary goal of the GBIF is to link biodiversity databases from around the world into one easily accessible network. Dissemination of biodiversity information is necessary for establishing the benefits of studying and conserving natural resources so that they can be used sustainably to take advantage of their immense economic, medical, social, and scientific potential. Despite the vast potential that lies in these resources, every day, somewhere in the world, extinction claims 100 species of plants, animals, and microbes.

The Facility link on the homepage takes visitors to information about the GBIF's background, mission, and organization. All OECD countries have been invited to join the GBIF. To date 21 countries have become voting participants in the facility's activities, with 3 others pending full membership and 10 associate participants who agree to share their biodiversity data and to develop electronic methods for accessing it. Membership also includes nongovernmental groups such as the United Nations Environment Programme, BioNET-International, and the Expert Center for Taxonomic Identification.



The GBIF has prepared pages, under the Work Programmes link, describing each of its four key action areas: data access and database interoperability, electronic cataloguing of names of known organisms, digitization of natural history collection data, and outreach and capacity building. Activities

under these programs include developing and maintaining an electronic catalogue of names of known organisms to serve as a reference resource for standardizing taxonomic work, and compiling an electronic handbook on the best practices for preparing digital images of specimens to share worldwide.

The website also provides a collection of over 100 related websites, under Related Links, organized by seven categories. These include international conventions and agreements; biodiversity search engines, tools, and software; and biodiversity research institutions and collections. Partnerships that the GBIF has developed with 15 international and regional organizations are described on the Relationships pages. The GBIF works with these groups to avoid duplication of effort and to gain essential input on its programs from experts in related disciplines. Among these organizations are Species 2000, the International Working Group on Taxonomic Databases, the Global Registry of Migratory Species, the European Environmental Agency, and the Inter-American Biodiversity Information Network.

Information about the Ebbe Nielsen Prize, awarded by the GBIF, is also available. The prize provides \$35,000 for the chosen promising young researcher to do biodiversity work outside his or her country of residence for up to six months. It was awarded for the first time in 2002 to Nozomi Ytwo of Japan, developer of a new database model called Nomencurator that tracks the conception, change, and use of scientific nomenclature over time. —Erin E. Dooley

Exchanging Organic Goods

Opening in early 2003, The Ethical Exchange Management Company, based in London, will be the first international commodities exchange in the world devoted entirely to organic goods.

Although open to producers from all countries, it is expected that small producers in developing countries will especially want to take part, as they have few other affordable means of making their products available to international outlets. Backers hope the exchange will reduce the influence of large retailers over organic pricing; such retailers have been accused of driving down prices. Some 500 producers are expected to participate as the exchange opens. In organizing the exchange, planners have focused on ensuring that all products traded will comply with the same import/export requirements as conventional produce.



Mexico to Ban Tobacco Ads

To help reverse a trend that could result in up to half a million tobacco-related deaths in Mexico before the end of 2010, Mexican health secretary Julio Frenk announced in June 2002 that tobacco company advertising will be banned from radio and television beginning in 2003. Tobacco companies have also agreed to print health warnings that take up 25% of the label on cigarette packs. Smoking is currently linked to five deaths per hour in Mexico and costs the government nearly \$3 billion—or 6–12% of its annual health care budget—in treatment costs for smokers with health problems related to their habit.

Chef's Special: Green Cuisine

More than 1,000 chefs and other food service providers in the United States have joined together to promote what they call "sustainable cuisine," proving that incorporating environmentally responsible practices into a business plan can still make for a successful restaurant. The Chefs Collaborative began in 1993 as a means of networking for local food producers and chefs, but has expanded to promote not only sustainable foods of all kinds—such as organic produce—but also sustainably produced furnishings, building materials, and energy choices for restaurants.

The collaborative also advises members on ways to trim costs in other areas so they can more profitably put locally produced foods on the menu.

